# Laboratory Quality Assessment of Canadian Atlantic Squid

(ILLEX ILLECEBROSUS)

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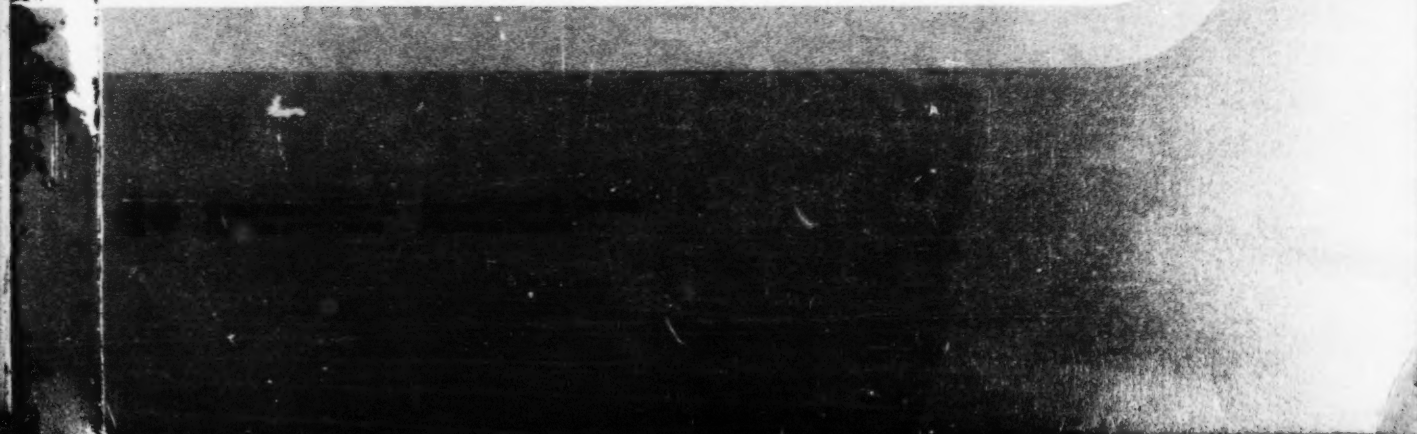

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# LABORATORY QUALITY ASSESSMENT OF CANADIAN ATLANTIC SQUID (*ILLEX ILLECEBROSUS*)

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## ABSTRACT

A. D. Woyewoda and P. J. Ke. 1979. Laboratory Quality Assessment of Canadian Atlantic Squid (*Illex illecebrosus*). Fisheries and Marine Service Technical Report No. 902, pp.

Chemical objective methods for evaluating the quality of Canadian Atlantic squid (*Illex illecebrosus*) have been described. Total volatile bases (TVB) in squid mantle is recommended for assessing squid quality; TVB values of less than 30 mg N/100g are indicative of excellent quality, while values in excess of 45 mg N/100g indicate unacceptable product. The chemical tests for trimethylamine (TMA) and free fatty acids (FFA) can be used as secondary quality parameters for squid quality assurance. Various chemical results have been shown to correlate well with data from the organoleptic evaluations, and guideline standards in terms of TVB, TMA and FFA values have been proposed for grading squid quality. The recommended procedures have been employed satisfactorily in our squid development studies.

## RÉSUMÉ

On a décrit des méthodes chimiques objectives servant à évaluer la qualité du calmar de l'Atlantique canadian (*Illex illecebrosus*). On recommande d'utiliser la valeur des bases volatiles totales dans le manteau pour évaluer la qualité du calmar; une valeur de moins de 30 mg N/100 g indique une qualité excellente, une teneur supérieure à 45 mg N/100 g un produit inacceptable. Les tests chimiques de la teneur en triméthylamine et en acides gras libres peuvent servir de paramètres secondaires pour vérifier la qualité du calmar. Les divers résultats des analyses chimiques semblent être en corrélation avec les données provenant des évaluations organoleptiques, et on propose des normes fondées sur les teneurs en bases volatiles totales, en triméthylamine et en acides gras libres pour classer le calmar en fonction de sa qualité. Les méthodes recommandées ont donné satisfaction au cours des études sur le calmar.

## INTRODUCTION

Canada's principal foreign customers to date have relied primarily on external color for quality evaluation of squid. Although convenient in the short term this practice has precluded the establishment of a grading system for Canadian squid (*Illex illecebrosus*) and in fact has led to confusion over exactly which criteria, besides color, could be applied for the purpose.

Squid color is prone to manipulation and can have little relation to final sensory attributes (Tanikawa, *et al.*, 1970; Ke *et al.*, 1979). In fact, maintenance of so-called "desirable" dark brown color in squid depends predominately on oxygen availability and holding temperature. The final color is a function of contraction and relaxation of muscles attached to pigment filled chromatophores; muscle relaxation renders the squid white in appearance. Squid maintained in chilled seawater (CSW) with aeration, well circulated refrigerated seawater (RSW), or in non-contact ice (NCI) will retain acceptable color for at least 3 days (Ke *et al.*, 1979; Botta *et al.* 1979). Therefore, color-independent factors should be employed for setting up quality grades.

When considering grading parameters for any food commodity the first attributes which come to mind are the gross characteristics of size, shape, color, odour and perhaps texture-features which register almost automatically on initial exposure to the product. Subsequent examination then reveals information at a more discriminating level such as deformities, discoloration, bruises, evidence of spoilage and the presence or absence of parasites. Final acceptance, since dealing with food, should however depend on sensory attributes determined by a trained taste panel. Such an evaluation is unfortunately a time and labour intensive operation requiring the training of panelists, repeated sampling, and statistical evaluation of results. Therefore we suggest the adoption of a practical alternative to eliminate the costly time and human error factors of the procedure, namely: the indexing of sensory data to chemically derived parameters.

### Biochemical Characteristics of Squid

In the laboratory, squid may be considered a biological-chemical entity. The mantle is composed of about 80% water, 18% protein, 1% fat, and traces of inorganic salts and minerals (Table I). The first component, water, is the life giving substance surrounding and suspending other molecules and acting as the medium in which biochemical reactions take place. Removal of this phase, as in the drying process, preserves the product by effectively stopping or greatly retarding deleterious reactions. The fat content of squid is very low and although fat oxidizes during storage to produce off-flavours, the overall effect of lipid deterioration on squid quality can be considered minimal. The remaining component, therefore, which is responsible for the decrease of quality is protein.

Protein is an ordered array of very large molecules and predominately of structural function. However, one protein class, the enzymes, is very active in life-sustaining biochemical reactions. The delicate state of enzyme equilibrium and control maintained by body regulators fails when an organism dies and from that point enzymatic reactions proceed unhindered. Since the circulatory system has stopped, raw materials for building are quickly depleated and only degradative reactions continue; structural proteins are reduced to smaller subunits by their "molecular cousins", the enzymes. Not only are these by-products foul smelling and putrid to the taste, but in squid some may produce undesirable discolorations. In some fish, particularly herring and mackerel, digestive enzymes of the viscera can literally digest their way through the belly flap (Takahashi, 1956). This extreme case of enzyme action is termed "belly burst".

Naturally occuring enzymes are not the only sources of degradative activity in stored squid. While living, all organisms have protective mechanisms barring the entrance of bacteria. When life processes stop, bacteria quickly invade the body and secrete powerful enzymes to begin the digestion of tissues. Therefore at death squid enters a state of uncontrolled enzymatic protein degradation from both natural and bacteria sources (Tanikawa *et al.*, 1970) which results in the accumulation of peptides, amines, ammonia and other nitrogen containing compounds (Takagi *et al.*, 1977). While this chaotic state is undesirable for preservation, a study of the levels of these by-products makes it possible to develop methods for the assessment of squid quality.

Although the fat content of squid mantle is low (less than 1%) and is not likely to influence the short term storage quality, squid liver is very high in fat, approximately 30%. Since fats are susceptible to enzymatic and chemical oxidation reactions, liver fat composition is greatly altered during iced and frozen storage of squid (Shchenikova, 1972). This factor may not specifically affect eating quality but is a useful phenomenon from a quality monitoring standpoint.

### Recommended Laboratory Tests for Squid Quality Assessment

An investigaton of chemical tests applicable to squid quality assessment was made by this laboratory. Potential procedures measuring chemical constituents such as volatile nitrogen compounds (Takagi *et al.*, 1971; Tozawa and Amano, 1969), proteins and selective amino acids (Tsuchiya *et al.*, 1977; Matsumoto, 1958), lipid compounds (Shchenikova, 1972, Jangaard and Ackman, 1965), carbonyls (Kim *et al.*, 1974) and phosphorous compounds (Saito *et al.*, 1960) were evaluated. According to the requirements of sensiti-

vity, simplicity, and reliability, three tests, namely: total volatile bases (TVB), trimethyl amine (TMA), and free fatty acids (FFA) were adapted to monitor changes in squid quality during various holding procedures. These methods together with a sensory evaluation guide appear in this report as Appendices A to D.

Quality changes of squid samples held at 2°C in terms of TVB, TMA, and FFA determined by the described methods have been summarized in Fig. 1,2, and 3, respectively. The broken lines indicate suggested limits for grade A (excellent) and grade B (acceptable) quality, and have been established from sensory data. TVB changes in squid by various handling methods at 2°C were also compared in Fig. 4.

Our preliminary data indicates a potential third indicator of fresh squid quality based on the accumulation of products of enzymatic fat hydrolysis, i.e. the measurement of free fatty acids (FFA). Squid liver has a fat content of about 30% and contains a high portion of triglycerides and unsaturates (Jangaard and Ackman, 1965). The increase of FFA in both liver tissue and liver oil as a function of holding time is depicted in Figure 3. However, the small overall change and larger deviation may limit the usefulness of this analysis. A more extensive study must be completed before FFA data can be applied objectively to the assessment of overall squid quality.

Guidelines for Quality Evaluation

Application of both parametric two-way analysis of variance (Larmond, 1977) and Friedman non-parametric (Siegal, 1956) statistics to organoleptic analysis of squid quality has provided satisfactory correlation with TVB and TMA data. As in previous reports (Ke *et al.*, 1979 a & b), food grade squid has been divided into two classes: grade A of excellent quality, and Grade B, of acceptable quality. The A,B, division is the point at which the taste panel first is able to detect a quality change in the product at $P \leq .05$ by non-parametric statistical treatment. Third class or grade F is of unacceptable quality and is rejected by the panel at $P > .05$ by parametric analysis. These grades are compatible with physical grading schemes based on mantle discoloration with grade B exhibiting slight yellow staining.

Using this system we suggest corresponding chemically derived limits as shown in Table 2. Grade B and F squid have lower TVB limits of 30 and 45 mg total volatile nitrogen per 100 g of mantle respectively. Similarly TMA values are set at <3, 3 to 10, and >10 mg TMA-N/100 g mantle for excellent, acceptable,and rejection quality. Free fatty acid levels have been tentatively set at <80 µmole/g mantle tissue and <300 µmole/g liver fat for excellent quality.

In summary, the quality assessment of Canadian squid can occur on several levels, namely: physical, sensory or chemical depending on the precision required and the time and facilities available for the evaluation. If external squid color is maintained through correct handling procedures then grading of physical chracteristics at dockside will for the most part be sufficient. However, in borderline situations, in cases of dispute, or for more involved storage studies, chemical assessment will be a pre-requisite. The described tests which are relatively simple, require minimal equipment, and provide reliable results will be of value for the quality assessment of Canadian Atlantic squid.

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TABLE 1. Approximate Content of Moisture and Fat in Squid

| Sample | Whole | Mantle* | Liver* |
|---|---|---|---|
| Weight, g | 190-340 | 110-170 | 16-27 |
| Length, cm | 45-62 | - | - |
| % Water | 78 | 80.3± 1.0 | 40.5±3.0 |
| % Fat | 1.6 | 0.5±0.2 | 31.1±2.6 |

*Data obtained from 30 samples.

TABLE 2. Recommended Chemical Guideline Values for Assessing the Quality of Squid. (The results have been correlated with sensory analysis).

| | Excellent (A) | Acceptable (B) | Unacceptable (F) |
|---|---|---|---|
| Total Volatile Bases (TVB) (mg N/100g mantle) | <30 | 30-45 | >45 |
| Trimethyl Amine (TMA) (mg N/100g mantle) | <3 | 3-10 | >10 |
| Free Fatty Acids (FFA) (µmole/g liver tissue) | <80 | - | - |
| (µmole/g liver fats) | <300 | - | - |

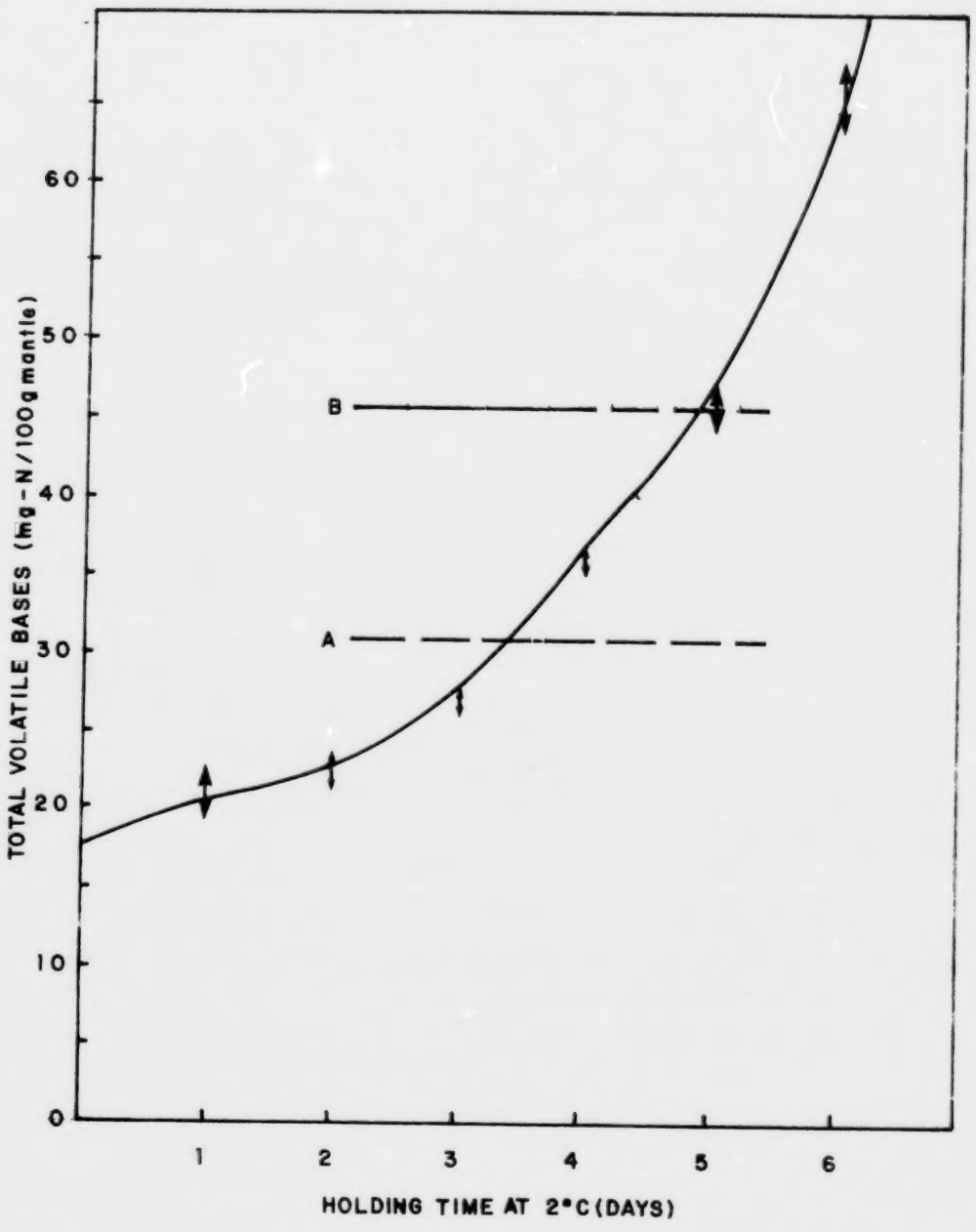



Figure 1. Changes in total volatile bases (TVB) over time for squid held in air at +2°C.

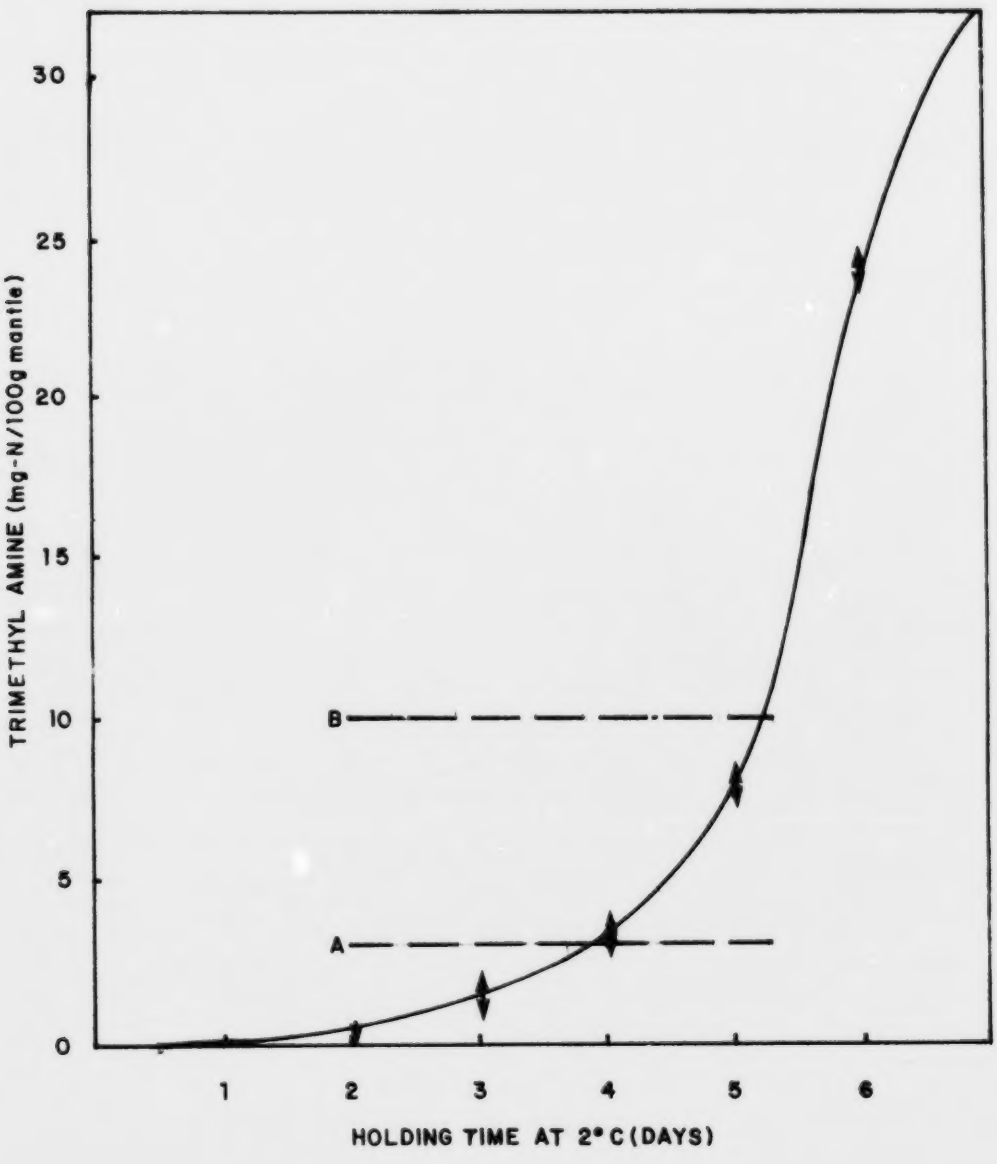



Figure 2. Changes in trimethyl amine (TMA) over time for squid held in air at +2°C.

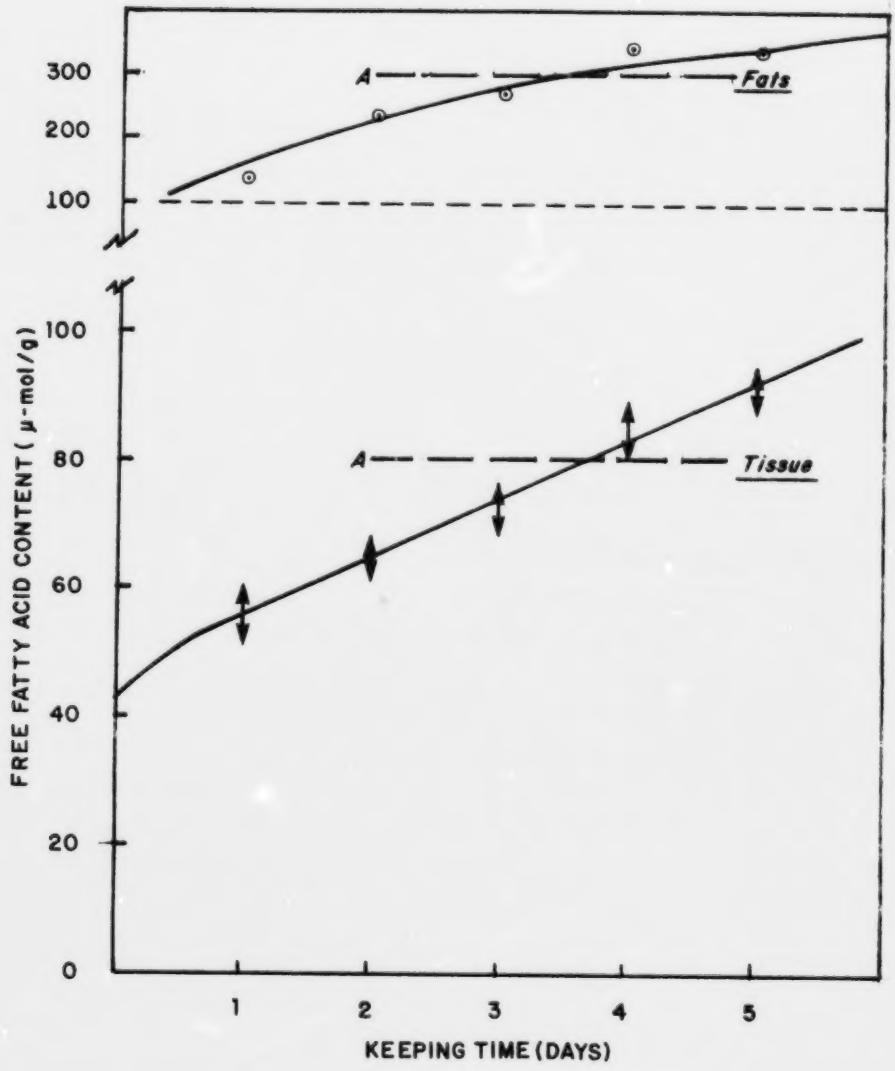



Figure 3. Changes in free fatty acid content (FFA) in liver tissue and liver fats over time for squid kept in air at +2°C.

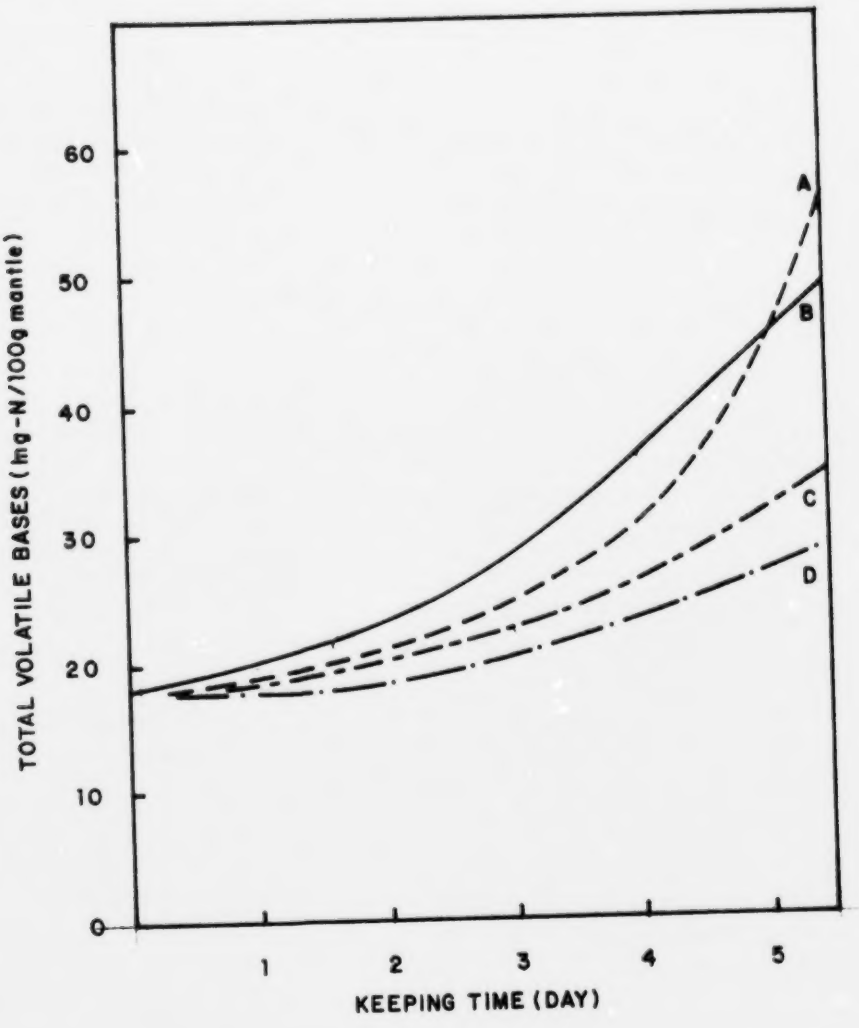



Figure 4. Changes in total volatile bases (TVB) over time for squid kept in sea water at about 2°C (Curve A), air (Curve B), non-contact ice (NCI) (Curve C), and chilled sea water (CSW) (Curve D).

## APPENDIX A

### Estimation of Total Volatile Bases (TVB) in Squid
### (Adapted from Cox and Pearson, 1962)

Reagents:

Boric acid solution (2%). Dissolve 2g boric acid in 100 ml $H_2O$.

Sulphuric acid 0.050N. To 1 litre $H_2O$ add 1.39 ml conc $H_2SO_4$. This solution must be standardized against standard NaOH solution.

Screened methyl red indicator. To 100 ml ethanol add 0.05g methyl red and 0.075g bromo cresol green.

Magnesium oxide

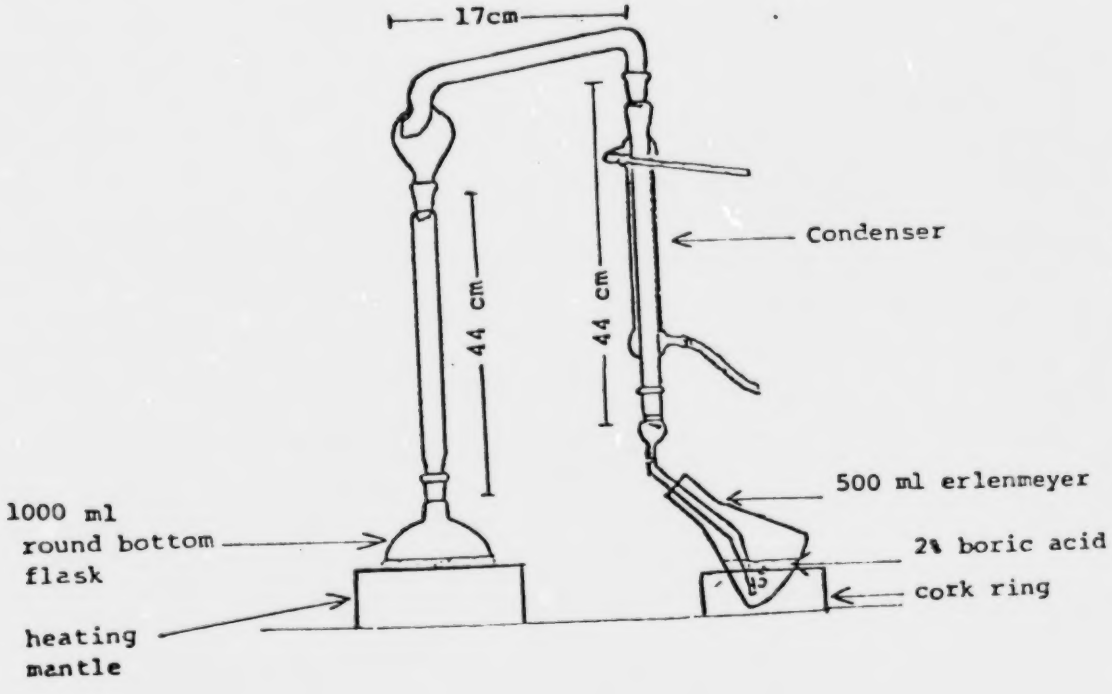
Anti-bumping granules (BDH)

Anti-foam silicon preparation

Apparatus:

Distillation apparatus or distillation assembly shown below:

Distillation Assembly for TVB
(dimensions approximate)



Procedure:

1. Blend squid mantles in Cuisinart food processor or blendor to fine paste.
2. Weigh 10g samples and freeze until ready to extract.
3. To 1000 ml round bottom distillation flask add 10g sample and 300 ml $H_2O$. Blend by inserting Polytron probe into flask.
4. Then add anti-bumping granules and 2g magnesium oxide.
5. Connect to still.
6. To 500 ml erlenmeyer receiving flask add 25 ml of 2% boric acid and a few drops of indicator.
7. Install receiving flask such that the receiver tube dips below the boric acid solution.
8. Heat the distilling flask so that liquid boils in exactly 10 minutes.
9. Using the same rate of heating, distill for exactly 25 minutes.
10. After distillation titrate the solution in the receiver flask back to the original color.

Calculation:

$$\text{TVB (mg-N/100g sample)} = \frac{(Vs - Vb \text{ mls Blank}) \times N \times 100 \times 14}{S(g)}$$

Where Vs, $V_b$, N and S denote the volume (ml) of titrant used for sample and blank, normality of $H_2SO_4$, and weight of sample respectively.

Example:

Squid, 10g, was analyzed for TVB. In the titration 2.75 ml of 0.053N $H_2SO_4$ was used. Blank was 0.02 ml.

$$\text{TVB} = \frac{(2.75 - .0.02) \times 0.053 \times 100 \times 14}{10}$$

$$= \frac{2.73 \times 0.053 \times 100 \times 14}{10}$$

$$= 20.2 \text{ mg N/100g}$$

or 20.2 mg-N%

## APPENDIX B

### Trimethylamine (TMA) Determination for Squid
### (Adapted from Dyer, W.J., 1945; Tozawa, *et al.*, 1971)

Reagents:

Stock TMA Standard (10.0 mg TMA-N/ml). Dissolve 0.682g TMA-HCl (dried overnight in desiccator) in $H_2O$. Dilute to 100 ml. Refrigerate.

Working TMA Standard (10.0 µg TMA-N/ml). Dilute stock 1→100.

10% formaldehyde (HCHO). Dilute 26.8 ml formalin (37.3% HCHO) to 100 ml with $H_2O$.

25% KOH. Dissolve 25g KOH in 75 ml $H_2O$. Cool.

7.5% trichloroacetic acid (TCA). Dissolve 7.5g TCA in 92.5 ml $H_2O$.

Moisture free toluene. (Dried over anhyd. $Na_2SO_4$). No need to distill.

Anhydrous Sodium Sulfate

Picric acid reagent. Stock. Dissolve 2.0g picric acid (dried overnight in desiccator) in moisture-free toluene.

Dilute to 100 ml with toluene.

Working Picric acid reagent. Dilute stock picric 1→100 with toluene.

Apparatus and Equipment:

1. Blender with small blender jar.
2. Low speed centrifuge (optional).
3. Glass funnels and filter paper Whatman #4 or glass wool.
4. Erlenmeyers, 50 ml.
5. Screw cap (teflon lined) culture tubes, 20 x 150 mm.
6. Rotator (eg. Cole Palmer Model 7621-20).
7. Visible range spectrophotometer set at 410 nm.

Appendix B (continued)

Procedure:

A. Preparation of Squid Extracts

1. Blend squid mantels in Cuisinart food processor or blender to fine paste.

2. Weigh 10g samples and freeze until ready to extract.

3. Add 10g sample and 20 ml of 7.5% TCA (1:2 extraction) to small blender jar and blend thoroughly.

4. Centrifuge at 4 C for 15 minutes at about 2000g (approx. 4000 rpm).

5. Decant supernatant fluid through glass wool. An alternate purification procedure to centrifugation and decanting is filtration through Whatman number 4 filter paper.

6. Extracts may be frozen at -20 C for extended periods of time with little deterioration.

B. Analysis of TCA Extracts

1. Pipet aliquot (1 - 4 ml) of extract into a 20 x 150 mm screw cap culture tube. Concentration of TMA in extract will determine appropriate aliquot size. For standard curve use 1.0, 1.5, 2.5, and 3.0 ml of working standard solution. Add water to total volume of 4.0 ml for both extracts and standards. For a blank use 4.0 ml $H_2O$.

2. Then add 1 ml 10% formaldehyde, 10 ml toluene, 3.0 ml 25% KOH.

3. Mix for 15 min. on rotator.

4. With pasteur pipet remove >5 ml of upper toluene layer to a large drying tube containing approximately 0.3 to 0.4g anhydrous $Na_2SO_4$. Shake gently until solution is clear.

5. Pipet 5 ml of picric acid working reagent into clean test tubes - if color change is evident when reagent is added to empty tubes, contamination is present and clean test tubes should be obtained.

6. Pipet 5 ml of toluene solution (from step 4) into tubes containing picric acid reagent and mix by swirling gently.

7. Measure absorbance at 410 nm.

Calculations:

$$\text{A. TMA-N} = \frac{\text{Conc. std } (\mu\text{g-N/ml})}{10} \times \frac{\text{Abs. sample}}{\text{Abs. std}} \times \frac{\text{ml std}}{\text{ml sample}} \times \left[ \frac{\text{ml TCA added}}{\text{wt}} + \frac{\text{wt} \times \% \text{ M}/100}{\text{wt}} \right] \quad \text{- - - - - - - - - - [1]}$$

where Conc. std = concentration of standard in µg-N/ml,

Abs. = absorbance of standard or sample

% M = moisture content of squid expressed in percent

wt = weight of squid used in extraction (expressed in grams)

B. Assuming a 10 g-N/ml working TMA-N std is used, then the equation can be simplified:

$$\text{TMA-n (mg N/100g)} = \frac{\text{Abs. sample}}{\text{Abs. std}} \times \frac{\text{ml std}}{\text{ml sample}} \times \frac{\text{ml TCA added} + [\text{wt} \times \% \text{ M}/100]}{\text{wt}} \quad \text{- - - - - - - - - - [2]}$$

Example:

Squid extract was produced by adding 10g of squid at 80% moisture to 20 ml of 7.5% TCA, blending and filtering. Upon analysis the following data was obtained.

| Solution | Volume | Absorbance |
|---|---|---|
| std | 1 ml | 0.1643 |
| sample | 3 ml | 0.0969 |

The TMA content of the sample is: by equation [2]

$$\frac{0.0969 \times 1}{0.1643 \times 3} \times \frac{20 + (10 \times .8)}{10}$$

$$= \frac{0.0969}{.4929} \times \frac{28}{10}$$

= 0.55 mg-N/100g squid

## APPENDIX C

### Free Fatty Acid Determination in Squid Liver
### (Adapted from Ke and Woyewoda, 1978)

Reagents:

Sodium Hydroxide 0.05 N. To 1 litre $H_2O$ add 2.0 gm NaOH. This solution must be standardized against a standard acid such as potassium hydrogen phthalate. Pre-standardized 0.05 N (N/20) NaOH is available from Fisher Scientific Co.

Aqueous meta-cresol purple indicator (0.5%). To 50 ml $H_2O$ add 0.5g m-cresol purple indicator. With stirring add 0.1 N NaOH dropwise until indicator just dissolves and maintains a strong violet color. Make up volume to 100 ml with $H_2O$. Test indicator by adding 5 drops to a mixture of 50 ml chloroform, 25 ml methanol, and 50 ml 2-propanol. A yellow solution will result which should change to violet upon addition of no more than 4 drops of 0.05 N NaOH. Adjust indicator with weak NaOH or HCl solution so that this criterion is met.

Methanol

Chloroform

2-propanol (isopropanol)

Anhydrous sodium sulfate

Apparatus:

1. Small blender (eg., Virtis)
2. Erlenmyer flasks - 250 ml
3. Separatory funnels - 250 ml
4. Micro - burette - 10 ml
5. Small buchner funnel assembly (11 cm)
6. Volumetric flasks - 100 ml
7. Aluminum drying dishes (disposable)

Procedure:

1. Excise livers from three or four squid by removing all surrounding membranes. Livers must be kept cool.
2. Rinse with distilled water and pat dry with lab tissue.
3. In beaker or other suitable container manually macerate livers with spatula.

4. With the aid of two spatulas remove membranes from the solution so that only a viscous liquid remains.

5. Pre-weigh 5g aliquots in small containers (eg. polycons from Cole Palmer International) and freeze until ready to analyze.

6. To blender jar add 5g liver, 50 ml chloroform and 50 ml methanol. Blend 1 minute.

7. Filter on buchner funnel through Whatman #4 filter paper and rinse with small amount of chloroform.

8. Add 45 ml $H_2O$ to filtrate and transfer to 250 ml separatory funnel. Rinse flask with chloroform. Flush with nitrogen, stopper and leave 2 or 3 hours - or preferrably overnight.

9. After equilibrium, using a regular funnel, slowly filter the lower chloroform layer through a double 11 cm filter paper (#4 Whatman inside and #1 outside) half filled with anhydrous sodium sulfate into a 100 ml volumetric flask. Rinse with with chloroform but do not exceed mark of volumetric. Fill to mark with chloroform.

10. Pipette 20 ml of chloroform filtrate into pre-weighed aluminum drying dish and allow solvent from dish to evaporate in fume hood. When completely evaporated place dish in vacuum oven at 35°C and connect to vacuum pump until completely dried (approx. 2 hours). Remove and weigh accurately.

11. Transfer remaining volumetric solution to 250 ml erlenmeyer. Rinse with 10 ml chloroform.

12. Then add 80 ml 2-propanol, 40 ml methanol and 12 drops meta-cresol purple indicator.

13. Titrate to violet endpoint with 0.05 N NaOH.

Calculations:

A. Fat content of squid liver may be determined from the formula.

$$\% \text{ fat} = \frac{(D2 - D1) \times V2}{V1 \times W} \times 100 \quad - - -[1]$$

Where: D1 = weight empty drying dish in grams
D2 = weight of drying dish with fat residue in grams
V1 = volume of aliquot in ml.
V2 = volume of volumetric in ml.
W = weight of squid liver in grams

Example 1:

If 5 gm of squid liver, a 100 ml volumetric, and a 20 ml aliquot are used, formula 1 becomes:

$$\frac{(D2 - D1) \times 100}{20 \times 5} \times 100$$

$$\% \text{ Fat} = \frac{(D2 - D1)}{1} \times 100 \text{ - - -[2]}$$

B. Free fatty acid content of squid liver expressed as μ mole/gm of liver tissue.

$$\text{FFAT} = \frac{1000\ N \times (V3 - Bk)}{W - (\frac{V1 \times W}{V2})} \mu \text{ mole g}^{-1} \text{ liver - - -}$$

Where V3 = ml titrant used, and

Bk = ml titrant for blank

Example 2:

If V1 = 20 ml, V 2 = 100 ml, and W = 5g

then formula [3] simplifies to:

$$\text{FFAT} = \frac{1000 \times N \times (V3 - Bk)}{5 - (\frac{20 \times 5}{100})}$$

$$\text{FFAT} = 250 \times N \times (V3 - Bk)\ \mu \text{ mole/g liver - - -[4]}$$

C. Free fatty acid content of squid liver expressed as μ mole/gm of liver fat.

$$\text{FFAF} = \frac{\text{FFAT} \times 100}{\% \text{ fat}} \text{ - - -[5]}$$

Example 3:

Under the condition of example 2, free fatty acid content of squid content of squid liver expressed as μ mole $g^{-1}$ fat may be calculated by combining equations [2] and [3] as:

$$\text{FFAF} = \frac{250 \times N \times (V3 - Bk)}{100 \times (D2 - D1)} \times 100$$

$$= \frac{250 \times N \times (V3 - Bk)}{(D2 - D1)} \mu \text{ mole g}^{-1} \text{ fat [6]}$$

Example 4:

In an analysis the following conditions were used:

| | |
|---|---|
| D1 = 1.302g | V3 = 8.67 ml |
| D2 = 1.593g | Bk = 0.02 ml |
| V1 - 20 ml | W = 5g |
| V2 = 100 ml | N = 0.052 N |

(a) % fat from formula [2] is:

$$[1.593 - 1.302] \times 100$$

$$= 29.1\% \text{ fat}$$

(b) FFAT per gm liver from formula [4] is:

$$250 \times 0.052 \times (8.67 - 0.02)$$

$$= 250 \times 0.052 \times 8.65$$

$$= 112.4 \ \mu \text{ mole } g^{-1} \text{ liver}$$

(c) FFAF per gm fat from formula [5] is:

$$\frac{112.4}{29.1} \times 100$$

$$= 386 \ \mu \text{ mole } g^{-1} \text{ fat}$$

Alternately from formula [6] FFA per gram of fat is:

$$\frac{250 \times 0.052 \times (8.67 - 0.02)}{(1.593 - 1.302)}$$

$$= \frac{250 \times 0.052 \times 8.65}{0.291}$$

$$= 386 \ \mu \text{ mole } g^{-1} \text{ fat}$$

## APPENDIX D

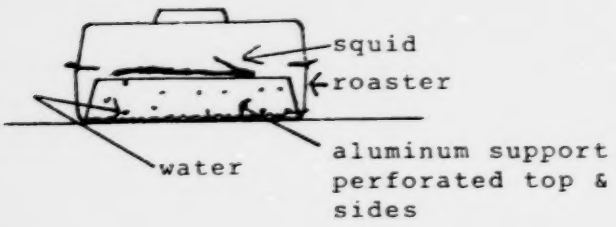
### Taste Panel Assessment of Squid Quality

(Adapted from Botta, *et al.*, 1979; Larmond, 1977; Siegel, 1956)

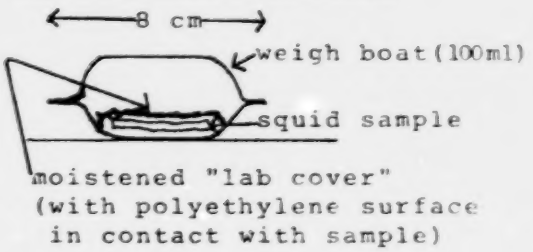
A. Materials and Equipment:

1. Enamel oval roasting pans (12" x 8") with aluminum inner supports (disposable trays approx. 2" high) , as shown below.
2. Sample presentation containers - eg., made from polystyrene (100 ml) disposable weigh boats (Canlab) - or other suitable container.
3. "Lab cover" from Fisher Scientific (14-206-30).
4. Styrofoam 4 oz. cups.
5. Distilled $H_2O$/7-up 1:1 at room temperature
6. Napkins

ROASTING ASSEMBLY




SAMPLE PRESENTATION CONTAINER




B. Preparation of Samples:

1. "Tube" squid by removing head, viscera, and cartilagenous pen. Remove skin manually.
2. Arrange squid on aluminum support in roaster (one roaster per treatment), add 1 cup cold water, cover, and heat in 375°F oven for 36 minutes.
3. While still hot, portion mantles into 3/4 inch rings and cut each in half.
4. Serve samples warm. A suggested container assembly is shown above. The moistened "Lab cover" helps retain moisture and heat in the sample.

5. Label the control sample "reference" and the others with random 3 digit numbers. One coded sample should be from the same animal as the reference each day to lend continuity to the panel and aid in the statistical treatment of the data. Coded samples should be presented in random order to minimize "order" effects.

6. Each panel booth should contain:

   a. one sample labelled "reference"
   b. coded samples
   c. one glass distilled water
   d. one glass 7-up/distilled water mixed (1:1) at room temperature.
   e. opaque bowls (eg. 4 oz. styrofoam) for expectoration of sample and rinse water
   f. napkins.

7. Panelists should be instructed to:

   a. evaluate coded samples in order presented
   b. complete entire sheet
   c. expectorate samples and rinse water if possible
   d. refrain from talking.

8. Minimum panel size should be 10 members.

C. Taste Panel Evaluation Form:

The panel sheet (following) has been drawn up by Dr. R. Botta, Fisheries and Oceans, St. Johns, Newfoundland. The descriptive terms may be changed for the individual group after evaluation of excellent and poor quality samples during preliminary round table sessions. The final descriptors used on the sheet should be acceptable to all panelists.

Panelists must check the category which best applies to the sample for each parameter of appearance, odour, flavour, texture, and overall acceptability. Although there is space on the sheet for four coded samples (one of which is to be the same as the reference), perhaps three samples should be considered sufficient for initial panels.

D. Statistical Treatment:

With this panel it will probably be sufficient to determine which treatment is significantly different from the reference sample. With a well trained panel, a 2-way analysis of variance such as that illustrated by Larmond (see references), pg. 32, may be used to determihe which samples differ from the control. If a difference is established, then Tukey's test (Larmond) is applied to determine which sample differs from the control (at the 95% level). Squid scored in the fourth category of the evaluation form was said to be of rejection quality.

With a panel which is not absolutely "expert", and which may not be completely in agreement with the supplied category descriptors, a non-parametric statistical treatment such as the Friedman 2-way analysis of variance (Siegel) may be most applicable. In this method the samples are judged according to rank or order, i.e., was the coded sample judged to be higher or lower quality than the disguished (coded) reference. If by this method the panel judges any sample statistically different from the coded reference (95% level), then that sample must indeed by exhibiting a decreased quality compared to the reference. This point may be considered the change from first to second grade quality.

The Friedman non-parametric 2-way analysis of variance is described below with the aid of an example.

1. Assign each quality category a number. For example in the "Odour" section, "fresh squid (sea water)" assign one, and to "Putrid", "Ammoniacal", "Repulsive" odour ..., five.

   See the following table:

   TABLE I. Raw Data Scores - Squid Overall Acceptability

| Code | 612 | 537 | 717 |
|---|---|---|---|
| Panelist | Sample | A(ref.) | B | C |
| 1 | | 1 | 4 | 4 |
| 2 | | 1 | 3 | 4 |
| 3 | | 1 | 2 | 3 |
| 4 | | 2 | 2 | 2 |
| 5 | | 1 | 2 | 1 |
| 6 | | 2 | 2 | 3 |
| 7 | | 2 | 2 | 3 |
| 8 | | 2 | 1 | 4 |

2. Raw scores of each sample must be ranked in order of magnitude compared to the coded reference ("A" in example) by assigning a "1" to the lower score and "2" to the higher. In the case of a tie, "1.5" is assigned to both reference and sample.

   TABLE II. Ranked Scores

| | Case 1 "B" Compared to "A" | | Case 2 "C" Compared to "A" | |
|---|---|---|---|---|
| Panelist | A | B | A | B |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 1 | 2 | 1 | 2 |
| 3 | 1 | 2 | 1 | 2 |
| 4 | 1.5 | 1.5 | 1.5 | 1.5 |
| 5 | 1 | 2 | 1.5 | 1.5 |
| 6 | 1.5 | 1.5 | 1 | 2 |
| 7 | 1.5 | 1.5 | 1 | 2 |
| 8 | 2 | 1 | 1 | 2 |
| SUM | 11.5 | 12.5 | 9 | 15 |

5. Label the control sample "reference" and the others with random 3 digit numbers. One coded sample should be from the same animal as the reference each day to lend continuity to the panel and aid in the statistical treatment of the data. Coded samples should be presented in random order to minimize "order" effects.

6. Each panel booth should contain:

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   c. one glass distilled water
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| 3 | | 1 | 2 | 3 |
| 4 | | 2 | 2 | 2 |
| 5 | | 1 | 2 | 1 |
| 6 | | 2 | 2 | 3 |
| 7 | | 2 | 2 | 3 |
| 8 | | 2 | 1 | 4 |

2. Raw scores of each sample must be ranked in order of magnitude compared to the coded reference ("A" in example) by assigning a "1" to the lower score and "2" to the higher. In the case of a tie, "1.5" is assigned to both reference and sample.

   TABLE II. Ranked Scores

| | Case 1 "B" Compared to "A" | | Case 2 "C" Compared to "A" | |
|---|---|---|---|---|
| Panelist | A | B | A | B |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 1 | 2 | 1 | 2 |
| 3 | 1 | 2 | 1 | 2 |
| 4 | 1.5 | 1.5 | 1.5 | 1.5 |
| 5 | 1 | 2 | 1.5 | 1.5 |
| 6 | 1.5 | 1.5 | 1 | 2 |
| 7 | 1.5 | 1.5 | 1 | 2 |
| 8 | 2 | 1 | 1 | 2 |
| SUM | 11.5 | 12.5 | 9 | 15 |

3. Total the ranks for each case. In the example, Case 1, "A" compared to "B", totals are 11.5 and 12.5 respectively. For "A" and "C", rank totals are 9 and 15.

4. Calculate Chi squared ($X^2$) from the formula:

$$Xr^2 = \left[\frac{12}{NK(K+1)} \sum_{j=1}^{k} (R_j)^2\right] - 3N(K+1)$$

where N = number of rows - eg. 8
K = number of columns - eg. 2
$R_j$ = sum of ranks in jth column - eg. 11.5 and 12.5 for case

$\sum_{j=1}^{k}$ = the sum of the squares of all $R_j$ values

eg. $(11.5)^2 + (12.5)^2$ in Case 1.

for the example, $X^2$ for Case 1 =

$$\frac{12}{8x2x(2+1)} \left[ (11.5)^2 + (12.5)^2 \right] - 3 \times 8(2+1)$$

$= (0.25 \times 288.5) - 72 = 0.125$

for Case 2: $X^2 = .25 \left[ (9)^2 + (15)^2 \right] - 72 = 4.5$

Therefore $X^2$ for Case 1, "A" compared to "B" is 0.125 and for Case 2, "A" compared to "C", $X^2 = 4.50$.

5. Test significance of $X^2$ from the table below which is reproduced from Siegel, page 249. If $X^2$ is greater than table value, then sample differs from reference with the indicated degree of significance.

Table of Critical Values

Probability under Ho that $X^2 \geq$ chi square

| df | .99 | .98 | .95 | .90 | .80 | .70 | .50 | .30 | .20 | .10 | .05 | .02 | .01 | .01 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | .00016 | .00063 | .0039 | .016 | .064 | .15 | .46 | 1.07 | 1.64 | 2.71 | 3.84 | 5.41 | 6.64 | 10.83 |

6. Conclusion, from the example

Case 1 Probability level for 0.125 is .80, i.e. 20%

Case 2 Probability level for 4.5 is .05, i.e. 95%

Therefore Sample "C" is significantly different from the reference (Sample "A"), but "B" is not. The 95% level, $P \leq 0.5$, should be used for determining significance with the Friedman method.

Tips for Successful Taste Panels

1. Panelists should discuss and agree on terminology during one or more round table discussions. They should be exposed to the highest and lowest quality which they will be expected to judge.

2. Taste panel room should be comfortable, preferably constant- i.e., not moved from room to room, and free of cooking odours.

3. Panelists should be physically divided from each other by barriers - either portable or permanent.

4. Panelists should be encouraged to complete sheets.

5. Panelists should be encouraged to expectorate samples and rinse water so as not to become over satiated by the product.

6. Absolute silence should be maintained during the panel - noise, particularly conversation, is distracting and will affect the results.

7. Choose panelists who will be available for most or all of the panels. A minimum of ten panelists should be maintained.

8. Treat panel members with respect - data will be much more consistent if the panel is comfortable and "happy".

## SENSORY ANALYSES OF SQUID

JUDGE_____ DATE_____

| FLAVOUR Sample Code | Fresh squid (sea-water) Flavour but almost bland. (Sl. sweet?) | Sl.squid flavour. Sl. cabbage flavour. Very slight after-taste. | Cabbage flavour. Sl. after-taste. | Sl. bitter, cheese-like flavour. after taste. | Very bitter. Very cheesy and offensive. Foul. Putrid and Sickening | COMMENTS |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| OVERALL ACCEPT-ABILITY Sample | Very Good | Good | Fair | Slightly Spoiled | Spoiled | COMMENTS |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

COMMENTS:

## SENSORY ANALYSES OF SQUID

JUDGE_____ DATE_____

| | | | | | | |
|---|---|---|---|---|---|---|
| ODOR<br>Sample Code | Fresh Squid (sea water) | Loss of fresh squid. Sl. rubbery & sl. cooked cabbage odor. | Stronger rubber & cooked cabbage odor. Sl stale. Sl. musty. | Unpleasant. Sl.Ammonical. Stale. Musty. Foul. Sl. sour. | Putrid. Ammonical. Repulsive Odor. Very musty. Strong. Rubbery. Strong stale odor. Very foul. | Comments |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| APPEARANCE<br>Sample Code | Off-white & sl. glossy. Surface smooth and firm. Good sheen | Creamy to yellowish. Sl. loss of sheen. Very slightly bruised. | Sl. sheen. Yellow to light brown. Sl. scabby & Sl. bruised outside. Sl. chalky & sl. curdy inside. | Light brown to dark brown Bruised and mod. scabby outside. Chalky & curdy inside. No sheen. | Pinkish brown. Blemished and scabby. Washed out. Bruised outside. Very chalky and very curdy inside. | Comments |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| TEXTURE<br>Sample Code | Smooth<br>Firm and rubbery (outside) spongy ~~(inside)~~ | Rubbery. Firm. Sl. chalky & sl. crumbly (inside). Sl. tough (outside) | Sticky & gluey (outside) chalky and crumbly (inside) | Sl.mushy (outside). Sl. blemished & scarred. Mushy (inside) and slimy. | Outside is mushy with very curdy interior. Very Crumbly. Highly blemished and scarred. | Comments |
| | | | | | | |
| | | | | | | |
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| | | | | | | |

COMMENTS: